Journal of Chemical Ecology, Vol. 31, No. 5, May 2005 (©2005)
DOI: 10.1007/s10886-005-5813-0

RAPID COMMUNICATION

(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-PENTACOSAPENTAENE, A KEY PHEROMONE COMPONENT OF THE FIR CONEWORM MOTH, *Dioryctria abietivorella*

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(Received April 8, 2005; accepted May 4, 2005)

Published Online May 13, 2005

Abstract—The sex pheromone of the fir coneworm moth consists of a blend of (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene and (9*Z*,11*E*)-tetradecadienyl acetate. Analogous blends of polyunsaturated, long-chain hydrocarbons with much shorter chain aldehydes or alcohols recently have been discovered in three other moth species in the superfamily Pyraloidea. These combinations of components from two distinct structural classes may represent an important and widespread new pheromone blend motif within the Lepidoptera.

Key Words—Sex pheromone, *Dioryctria abietivorella*, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene, (9*Z*,11*E*)-tetradecadienyl acetate, Pyralidae, Crambidae

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INTRODUCTION

Almost all of the female-produced pheromone blends from the more than 500 species in the lepidopteran suborder Ditrysia for which pheromones have been identified are composed of components with the same or similar chain lengths, structures, and volatilities (Ando et al., 2004; Witzgall et al., 2004). Furthermore, for a particular species, the blend components usually belong to only one of two general structural classes. The first class consists of C_{10} to C_{18} primary alcohols and their corresponding acetates and aldehydes, whereas the second consists of C_{17} to C_{23} polyunsaturated hydrocarbons and epoxides (Ando et al., 2004). Exceptions to this pattern are rare; the pheromone of the tomato fruit borer, *Neoleucinodes elegantalis* (Crambidae: Pyraustinae), a blend of (*E*)-11-hexadecenol and (3*Z*,6*Z*,9*Z*)-tricosatriene, is one such example (Cabrera et al., 2001).

In spring of 2004, we found that the pheromone blends of two pyralid moths, the navel orangeworm (NOW), *Amyelois transitella* (subfamily Phycitinae), and the meal moth, *Pyralis farinalis* (subfamily Pyralinae), contain components of dissimilar structural types. The major component of the NOW blend was identified as (11*Z*,13*Z*)-hexadecadienal (Coffelt et al., 1979), but this single component was only weakly attractive. Using wind tunnel bioassay-directed fractionations and coupled gas chromatography–electroantennography (GC-EAG), we discovered that (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-tricosapentaene and (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene are components of the NOW blend, whereas the meal moth blend contains (11*Z*,13*Z*)-hexadecadienal and (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-tricosapentaene (J.G. Millar, L.P.S. Kuenen, and J.S. McElfresh, unpublished data). Although the pentaenes elicit weak EAG responses, they are critical components of the attractants. The presence of the pentaenes in NOW has been independently verified (Leal et al., 2005).

The fir coneworm, *Dioryctria abietivorella* (Pyralidae: Phycitinae), causes substantial damage in coniferous seed orchards in western North America. The major component of its pheromone [(9*Z*,11*E*)-tetradecadienyl acetate, (9*Z*,11*E*)-14:Ac] had been known since 1985 (G. Grant, unpublished data), but, as with NOW, the single component was minimally attractive in field trials. Here, we report that (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene is a crucial component of the pheromone blend of the fir coneworm.

METHODS AND MATERIALS

Douglas fir cones infested with fir coneworms, collected near Chico, CA, were held in individual containers under ambient laboratory conditions. Emerging moths were segregated by sex and placed in a light box, 16:8 L:D, in an

environmentally controlled room. Pheromone glands of virgin females were clipped off ca. 6 hr after lights off and extracted in pentane. Extracts were analyzed by coupled GC-EAG on DB-5 and DB-WAX columns as previously described (McElfresh and Millar, 1999). An aliquot of a composite extract from 212 females was analyzed by GC-MS (HP-6890 GC interfaced to an HP 5973 mass selective detector, electron impact ionization, 70 eV), using splitless injections with a DB-5MS column programmed from 50 to 250°C at 10°C/min. A second aliquot was treated with 10 μl of a solution of 4-methyl-1,2,4-triazoline-3,5-dione (MTAD, 2 mg/ml in CH_2Cl_2), and the adduct was analyzed by GC-MS (injector 300°C; program 100°C/1 min, 10°/min at 300°C, hold 20 min).

(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-Pentacosapentaene was synthesized from (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosapentaenoic acid ethyl ester (Tokyo Kasei Kogo Co., Tokyo, Japan) by reduction with $LiAlH_4$, tosylation of the resulting alcohol, and alkylation with pentyl magnesium bromide with CuI catalysis (Jain et al., 1983). Isomerically pure (9*Z*,11*E*)-14:Ac was prepared by a new synthesis (J.A. Moreira and J.G. Millar, unpublished data). (*Z*)-9-14:Ac was obtained from Bedoukian Research (Danbury, CT, USA).

Field trials were conducted in a seed orchard near Vernon, BC, Canada, during August–September, 2004. Test compounds were loaded onto 11-mm gray rubber septa (The West Co., Lionville, PA, USA) in 100 μl hexane, with 5 mg/ml each of butylated hydroxytoluene (BHT) and Sumisorb 300 added to the hexane solutions as stabilizers. Doses tested are shown in Table 1. Baited sticky traps were hung from branches (~1.5 m above ground), and spaced ~25 m apart.

TABLE 1. TRAP CATCHES (MEAN ± SE) OF MALE *D. abietivorella* IN PHEROMONE-BAITED TRAPS IN A SEED ORCHARD NEAR VERNON, BRITISH COLUMBIA[a]

Lure blend			
(9*Z*,11*E*)-14:Ac (μg)	Pentaene[b] (μg)	(*Z*)-9-14:Ac (μg)	Mean trap catch
100	0	0	0
100	100	0	2.8 ± 0.9 c
100	500	0	51.4 ± 6.4 a
100	0	3.3	0
100	100	3.3	2.8 ± 1.1 c
100	500	3.3	27.6 ± 7.8 b
Blank			0

[a] Trial conducted from August 12–September 14, 2004, with five replicates counted weekly. Trap catch data analyzed by ANOVA followed by Student–Newman–Keuls (SNK) test. Means followed by different letters are different at $\alpha \leq 0.05$. Zero values were not included in the ANOVA or SNK analyses.

[b] Pentaene = (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene.

RESULTS AND DISCUSSION

GC-EAD analyses indicated strong and consistent antennal responses to two compounds in pheromone gland extracts (Figure 1, peaks 1 and 2). The major component was identified as (9*Z*,11*E*)-14:Ac (peak 2) by comparisons of retention times on DB-5 and DB-WAX GC columns, mass spectra, and EAG responses with those of authentic standards. The position of the diene was verified by diagnostic ions (*m/z* 194, base peak, *m/z* 336, 16%) in the mass spectrum of the MTAD adduct. The earlier eluting peak 1 was present in insufficient amounts to obtain a mass spectrum. However, its retention indices on DB-5 and DB-WAX columns matched those of (*Z*)-9-14:Ac, and comparisons with tabulated retention index values of all possible C14 monoene acetates confirmed the position and geometry of the double bond (Marques et al., 2000). However, blends of these two compounds attracted virtually no moths in field trials. In early 2004, our discovery of pentaene compounds in NOW extracts prompted us to carefully examine our composite coneworm extract (212 females) for similar compounds, resulting in the discovery of (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene (Figure 1, peak 3) in an approximately 1:1 ratio with (9*Z*,11*E*)-14:Ac. The identification was confirmed by exact

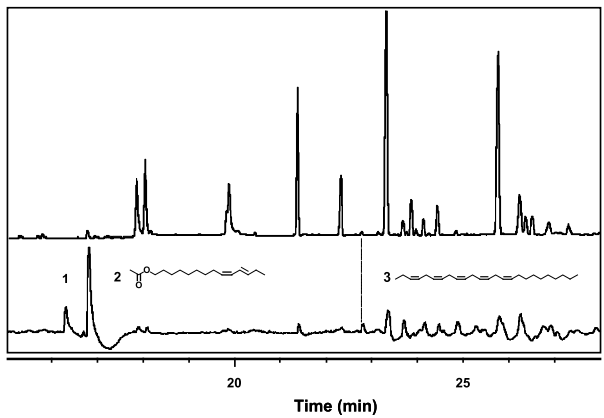

FIG. 1. Gas chromatogram (top trace) and corresponding electroantennogram trace from the antenna of a male *D. abietivorella* moth challenged with an aliquot of pheromone gland extract from female moths. Peak 1, (*Z*)-9-14Ac; peak 2, (9*Z*,11*E*)-14:Ac; and peak 3, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene. Other EAD responses were to straight- and branched-chain cuticular hydrocarbons. Conditions: DB-5 column (30 m × 0.25 mm ID, 0.25 μm film; 70°C/1 min, 10°/min–275/20 min. Approximately four female equivalents injected splitless.

matches of retention times with those of an authentic standard on polar and nonpolar columns and by its diagnostic mass spectrum characterized by even-mass fragments that unequivocally placed all the double bonds (Underhill et al., 1983, and references therein). In particular, cleavage between C_8 and C_9 with hydrogen transfer from C_5 gave a strong $C_2H_5(CH{=}CH)_3H]^+$ ion (*m*/*z* 108, 38% of base peak), whereas cleavage between C_{10} and C_{11} with hydrogen transfer from C_{14} produced the analogous $C_9H_{19}(CH{=}CH)_3H]^+$ ion (*m*/*z* 206, 9% of base peak) from cleavage and rearrangement from the other end of the chain. Because of the small amounts present, even in a composite sample from 212 moths, it has not been possible to verify that all the double bonds have the *Z* configuration, for example, by GC-FTIR. Nevertheless, the exact retention time and mass spectral matches, coupled with the strong biological activity (see below) provide strong evidence that the natural compound is the all-*Z* isomer.

(3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-Pentacosapentaene elicited unexpectedly weak responses from male moth antennae in GC-EAD analyses (Figure 1, peak 3). However, field trials demonstrated that this compound is a key component of the pheromone blend (Table 1). Although (9*Z*,11*E*)-14:Ac (Table 1) and (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-pentacosapentaene (second trial, data not shown) were not active as single components, a 5:1 ratio of the pentaene:acetate was attractive to male moths. When added to the 5:1 blend, (*Z*)-9-14:Ac decreased attraction at the rate tested, despite being present in pheromone gland extracts (Table 1).

These recent examples of pheromone blends from four related species in the superfamily Pyraloidea, consisting of one or more polyunsaturated long-chain hydrocarbons in combination with much shorter-chain aldehydes, alcohols, and acetates, suggest that these combinations represent a new and possibly widespread motif in lepidopteran pheromone chemistry. These examples also suggest a possible reason (i.e., missing long-chain components) why the pheromone blends of some other moths (e.g., the sugar cane borer, *Diatraea saccharalis* (Crambidae)) have proven so elusive.

These blends have other remarkable features. First, their dissimilar components almost certainly arise from two independent pheromone biosynthesis pathways, with the shorter chain components probably being synthesized in the pheromone gland and the longer chain hydrocarbons possibly being synthesized in oenocyte cells and transported to the gland through the hemolymph (see Jurenka, 2004). Second, it is unclear how calling female moths successfully emit optimal blends of compounds with such substantial differences in vapor pressure. Third, it is surprising, but not unprecedented (e.g., Cabrera et al., 2001), that the polyunsaturated hydrocarbon components elicit relatively weak responses from male antennae, despite their being crucial to eliciting behavioral responses. Answers to these and other questions may be forthcoming from ongoing explorations of these novel pheromone blends.

Acknowledgments—This project was funded by the Forest Genetics Council of the British Columbia Ministry of Forests and the USDA Forest Service. We thank Nancy Gillette for her support and encouragement to pursue this project, Amanda Roe for confirming moth species identifications, and Mariana Krugner for technical assistance.

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